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## **Measuring Baseline Cortisol Levels in Cetaceans: Developing a Novel Non-Invasive Analysis Method**

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### **LONG-TERM GOALS**

With this project, we propose the development of a novel tool in stress research; a non-invasive method to measure long-term cortisol levels in cetaceans. Cortisol in cetacean skin samples is expected to reflect levels of chronic stress, as the acute stress potentially caused by the sampling itself is not expected to enter this matrix for days or even weeks. Skin samples can thus provide information on the long-term physiological status of the animal.

### **OBJECTIVES**

At the conclusion of the project period, we expect to have provided a greater understanding of the potential of cetacean skin as a matrix for measuring cortisol, the basal levels and the minimum required sample size, and the effectiveness of various skin collection methods.

### **APPROACH**

The project will be executed under the PI Thea Bechshoeft, in collaboration with colleagues at Aarhus University (Professor Rune Dietz, Jonas Teilmann, PhD, Signe Sveegaard, PhD, PhD candidate Andrew Wright, and Dr. Christian Sonne, PhD), University of Copenhagen (assistant professor Martin Hansen, PhD, and associate professor Bjarne Styrrishave, PhD), and Fjord & Bælt Marine Research Centre (Magnus Wahlberg, PhD, and chief trainer Jakob Højer Kristensen, MSc).

The proposed study will develop a method for extracting cortisol from cetacean skin using a two-step inverse pressurised liquid extraction (PLE) technique modified from Hansen et al. (2011a). A clean-up procedure will then be implemented using aminopropyl solid phase extraction (SPE) cartridges followed by silica SPE clean-up (modified from Hansen et al. 2011b). The purpose of this clean-up is to remove lipids such as mono- di- and triglycerides, sterols, stanols, cholesterol, and fatty acids from the samples for better chromatographic quality and lower cortisol detection limits. Finally, the samples will be measured using a high pressure liquid chromatography–mass spectrometry (HPLC-MS) detector, using a deuterated cortisol analogue as internal standard. For the proposed method, the relevant criteria for determining the chromatographic quality will be determined including

repeatability, instrument repeatability, reproducibility, absolute recovery, relative recovery, limit of detection (LOD), and limit of quantification (LOQ). The aim is to fully develop a method sensitive enough to allow accurate determination of cortisol in cetacean skin samples. Since non-invasively collected skin samples from relatively small cetaceans may be as small as 500 mg or less, estimating the minimum sample size is important. The method will be developed using skin samples from the harbour porpoise (*Phocoena phocoena*).

The Section of Toxicology, Dept. of Pharmaceutics & Analytical Chemistry (under Associate Professor Bjarne Styris have) from the Faculty of Pharmaceutical Sciences at the University of Copenhagen, Denmark, will be in charge of the practical side of developing the proposed method for measuring cortisol in cetacean skin samples.

## **WORK COMPLETED**

Due to administrative complications, the lab work has only just commenced this August 2012, and all results are expected to be available before the end of the year. The end date of the project is March 15<sup>th</sup>, 2013.

## **RESULTS**

The lab work has only just commenced this August 2012, and all results are expected to be available before the end of the year. The end date of the project is March 15<sup>th</sup>, 2013.

## **IMPACT/APPLICATIONS**

A working method for assessing cortisol in non-invasively collected cetacean skin samples will bring new possibilities for stress assessment in cetaceans, opening up a new avenue of research in physiological response studies following exposure to stressors. This study represents the first real step toward establishing such a method.

## **REFERENCES**

- Hansen M, Jacobsen NW, Nielsen KN, Björklund E, Styris have B & Halling-Sørensen B. 2011b. Determination of steroid hormones in blood by GCMS/MS. *Analytical and Bioanalytical Chemistry*, 400: 3409-3417
- Hansen M, Krogh KA, Halling-Sørensen B & Björklund E. 2011a. Determination of ten steroid hormones in animal waste manure and agricultural soil using inverse and integrated clean-up pressurized liquid extraction and gas chromatography-tandem mass spectrometry. *Analytical Methods*, 3: 1087-1095